

The University of Chicago

A COLORIMETRIC STUDY OF GENIC EFFECT ON GUINEA-PIG COAT COLOR

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

Department of Zoology

1938

By

GERTRUDE HEIDENTHAL

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The University of Chicago, Chicago, Illinois

Received October 18, 1939

INTRODUCTION

ONE approach to the study of gene physiology is obviously a description of the end-products of genic interaction. Estimates of quantity and an elucidation of any qualitative differences between or within allelic series should aid in the establishment of a general theory and thus in an understanding of the links between gene and phenotype. With respect to pigmentation in the guinea-pig, it has seemed desirable to continue the attack started by RUSSELL (1939) on the quantitative aspects of the problem although the need for qualitative chemical analysis cannot be denied since, at the present time, we have only rather meager suggestions as to differences in kind. We know, for example, that in general the yellow pigments from mammalian hair are more readily soluble in cold dilute alkalis than are the sepia melanins, although information on other differences between these pigments, and possible variations within the sepia and yellow end-products are still lacking. WRIGHT's work (1927) with the Milton-Bradley color-wheel led him to the suggestion that the differences between red and yellow grades were more than simple quantitative variations since the proportion of orange required to match red skins (using black, white, yellow and orange) was greater than that needed for the yellow ones. The more recent work of DANIEL (1938) giving spectrophotometric measurements of solutions of sepia pigment from the hair of the mouse has indicated, however, that qualitative differences probably do not exist within the genotypes tested (combinations of the A, B, C series). That care must be exercised in drawing conclusions from these data is indicated by the author's statement to the effect that there is still some uncertainty concerning the method.

Although the quantitative measurement of hair pigments is fraught with difficulties, some rather satisfactory attempts have recently been made by EINSELE (1937) and DUNN and EINSELE (1938) for the mouse, and by RUSSELL (1939) for the guinea-pig. In the latter case, advantage was taken of an observation made by DURHAM (1904) and others that yellow pigment is rather readily soluble in cold, dilute alkali. RUSSELL, accordingly, was able to develop a colorimetric method for the determination of intensity differences among animals with yellow fur. Since the sepia pigments are relatively resistant to this treatment the same method could

not be used for this color quality. She therefore developed another technique by which the pigment was first separated from a known weight of hair, essentially according to the method of EINSELE, and then titrated with KMnO_4 to determine the amount of this substance which could be reduced by the isolated pigment. EINSELE, meanwhile, had found that the dark pigments of mouse hair could be made to dissolve by boiling in solutions of KOH (1937). The present investigation was undertaken for the purpose of extending the observations on the yellow series and of developing a colorimetric method for the sepia genotypes. It was also hoped that if the sepia pigments could be made to go into solution, the resulting color quality might be sufficiently similar to that of yellow to warrant quantitative comparisons between the two series. A colorimetric method for the sepia series has been developed, but the solutions were found to have a somewhat duskier hue than those of the yellow series. Thus comparisons between the two qualities are not available.

The author is grateful to PROFESSOR SEWALL WRIGHT for provision of the material and for help and guidance throughout the problem. Acknowledgment is also due to the Rockefeller Foundation for support of the colony.

MATERIALS AND METHODS

The major color-factors of the guinea-pig have been so thoroughly investigated by WRIGHT (1915, 1916, 1917, 1923, 1925, 1927) that it is hardly necessary to describe the stocks at length. Of the seven major series of alleles, readings were made on compounds of the members of four. These include *E*, *e* in which *E* governs the production of sepia, and *e* of yellow quality; the albino series *C*, *c^k*, *c^d*, *c^r*, *c^a* the members of which modify the intensity of both yellow and sepia; the *P*, *p* series in which the homozygous recessive dilutes sepia but has no effect on yellow; the *F*, *f* series in which *ff* reduces yellow but has no effect on sepia except in combinations involving *E-ffpp* in which case the animals are of a low grade of yellow or white. Practical considerations have made it impossible up to the present time to introduce the various combinations into a single isogenic stock although the desirability of this is evident.

The routine procedure in the color experiments was to grade each animal within a day or so after birth by means of two qualitatively different sets of pelts standardized according to graduated steps of intensity of yellow and sepia, respectively. Genotypes were assigned on the basis of color quality and intensity, the genotypes of the parents, and in some cases, breeding tests. Three to four weeks after birth, hair was clipped to approximately equal length of base from the mid-dorsal region of the body and stored in manila envelopes away from any intense source of light. The time between collection and use varied from a few weeks to a year.

The yellow series

The technique for the yellow genotypes was substantially that followed by RUSSELL. The hair was first washed in two changes of cold ether and dried. Samples weighing 100 mgs (weighed to the nearest mg) were then placed in 10 cc of a .5N solution of NaOH and allowed to stand for five days. The unhydrolyzed hair was removed by filtration and the resulting colored solutions were compared to each other in a colorimeter (Klett biometer). As explained by RUSSELL, the intensity of color seen in the field of a colorimeter depends upon the intensity of the colored solution and the height of the column through which the light passes. Furthermore, if two intensities are matched, $\text{intensity } 1 : \text{intensity } 2 = \text{height } 2 : \text{height } 1$. It follows from this relationship that if height 1 is fixed arbitrarily (for example, at 15 mm) and height 2 is determined by matching the intensity of solution 2 against that of solution 1, the ratio of these two heights will give a measure of the relative intensities of the two solutions.

Before a given series of comparisons was made, the colorimeter was calibrated by placing a portion of a given standard in each of the two colorimeter cups. These identical samples were read against each other in the manner described above. The colorimeter light was adjusted until it was possible to secure five consecutive readings in which height 2 fell within a range of $15 \pm .4$ mm (height 1 = 15 mm).

The determinations for a given animal were made as follows. The duplicate experimental solutions from a particular sample were first compared to each other by means of ten consecutive, independent readings. Then each was compared with one of a series of standards made by diluting an intense solution from the hair of a single yellow adult guinea-pig (grade 9 and constitution *eeC-F-*) with the .5N solution of NaOH in the proportions of 1:0, 2:1, 1:1, 1:2, 1:6. Thus for a given animal there were two comparisons with standard, each involving ten readings, hence ten ratios (h_1/h_2). The arithmetic average of the means of these two series of ratios was taken as the colorimetric value (CV) for the animal.

The only departure from the above procedure was in the case of grades 0 and 1, at the lower end of the intensity scale. Solutions from these grades were so light that the two sides of the colorimeter field were indistinguishable within a mean range of about 4 mm. The preparations were, however, not colorless but of a distinctly yellow tinge and it was therefore desirable to have at least an approximate value for them on the colorimetric scale. The practice was, accordingly, to use the lightest standard and with that to make four estimates each of the upper and lower limits of the indistinguishable band. The mid-point of this range was taken as the final determination.

The sepia series

The method for separating the sepia pigment from the keratin structure was essentially that developed by EINSELE (1937) and later used by RUSSELL. The keratin was hydrolyzed in 6N HCl and the resulting suspensions containing the pigment granules, were centrifuged and washed in water to separate the hydrolysate from the pigment (plus the small quantities of occluded impurities). For every animal in the present work two samples, each weighing 100 mgs, were treated in this manner. Considerable difficulty in separation was experienced with grades 10-12 but by prolonged and repeated centrifugation and careful slowing of the centrifuge, success was finally attained in four cases. These experiences agree in general with those of RUSSELL who circumvented the difficulty with centrifugation by adding weighed quantities of the coarsely granular pigment from black animals, to the suspensions, and was thus able to clear the hydrolysate, since the lighter pigments were precipitated by occlusion. The author did not use this method for the following reason. The data secured by RUSSELL on the percentage weight of melanin in sepia hair indicated that the proportion of pigment in these genotypes is very small (a mean of 1.37 per cent for the genotype $E-c^{dca}P-$). Thus for the lighter grades reliable weights could not be attained. The experimental error in adding extra black pigment for purposes of centrifugation might, therefore, tend to obscure the true values for the pale genotypes. This may account to some extent for the fluctuating permanganate values at the lower end of the scale.

A different procedure from that used by RUSSELL was followed after the technique of separation had been completed. The pigment (two samples from each animal) was put into solution by boiling for $1\frac{3}{4}$ to $2\frac{1}{2}$ hours in a reflux condenser with a .2N solution of KOH. The quantity of alkali was varied from 25 cc to 100 cc in accordance with the amount of pigment judged to be present on the basis of grade. This procedure was followed in order that all solutions might be of an intensity suitable for reading against a standard made by dissolving the pigment in 100 mgs of hair from an animal of grade 19 and constitution $E-c^{c}P-$, in 100 cc of the solution of KOH. The alkali was added directly to the pigment plus the small amount of water left after the last decantation. Consequent slight variation in pH could have had little if any effect on the readings since it will be evident from a later analysis that the errors between two samples from the same animal were small.

Preliminary work indicated that it was not always easy to tell exactly at what point the pigment went into solution. At times the "solution" would appear to be clear, yet filtration and centrifugation indicated that small particles were still in suspension. The practice, therefore, was to boil the suspensions for about one-half hour beyond the time when, to the naked

eye, they looked clear. In no case did subsequent filtration or centrifugation indicate any detectable, undissolved granules. Tests of additional boiling were made on 14 of these solutions. In each case, two samples of sufficient quantity to fill the colorimeter cups were read against each other. One of these was then saved and all of the remaining solution was boiled for an additional hour. Determinations for the portion which had undergone extra boiling were made against the sample which had been saved. An analysis by Student's method for paired comparisons yielded a value of 1.24 for t . This shows that no significant change in intensity had occurred. It is assumed, therefore, that the technique of boiling beyond the time when the suspensions appeared to have gone into solution had no appreciable effect, at least within the limits indicated.

After the solutions had been adjusted for volume, they were read against the standard ($E-c^cP-$, grade 19) and against each other. As in the yellows, each determination was taken as the mean of ten ratios secured from ten independent readings. The colorimeter value (CV) for a given animal is the arithmetic average of the two means secured from reading the experimental solutions against standard, except for the lower grades in which less than 100 cc of KOH was used. In these cases the average was multiplied by the appropriate factor, to give the true colorimetric value.

The possibility that changes in standard might have occurred was checked at the end of four months (three weeks after the last experimental determinations were made) by preparing two solutions from the same sample of hair which had been used in the preparation of the standard itself. These two solutions when compared with the original standard gave mean readings of 1.076 and 1.063. Such figures indicate that the standard probably had faded slightly in spite of the ordinary precaution of keeping it tightly stoppered in a photographic dark room, except when readings were being made.

A second test for the possible effects of change in standard on the readings was made by examining the determinations within grades 21, 20, 19, 18 (groups having the largest numbers) for trend. The data for each group were trichotomized according to the time when the experiments were performed, and a grand unweighted mean for each of the three periods was determined. These values were 1.137, 1.157, 1.189 for the first, second and third periods, respectively. The numbers are too small for an elaborate statistical study of trend, but the determinations for the three periods are at least consistent with the view that the standard did undergo a slight reduction in intensity. Such a change is not regarded as serious since it must have been small and it will be evident that the variability within both grade and genotype is relatively large.

RESULTS AND DISCUSSION OF RESULTS

Errors of the methods

Some measure of the reliability of the methods is necessary. The errors can be grouped into two classes: (1) the experimental error involved in sampling from the hair, weighing the hair and preparing the solutions for colorimetry, (2) the colorimetric error. The latter was determined directly, in the case of the readings of sample two against sample one, by finding the average variance within the sets of ten readings ($\sum_1^n \sum_1^{10} (v-m)^2/9n$) where n is the number of comparisons, the v 's are the individual readings and the m 's are the means of the sets of ten readings. In the case of yellow (101 cases, including all of grades 2-12 and two of grade one) this variance was .00090, indicating a standard deviation of .030. The standard error of the mean of ten readings was thus only .0095 ($= .030/\sqrt{10}$) or 0.95 percent, since the mean of the 101 means was 1.00.

The variance of set means (sample two read directly against sample one) was .00296 ($= \sum_1^{101} [m-1.00]^2/100$). This is compounded of the experimental errors in the preparation of both samples ($2\sigma_e^2$) and the colorimetric error (σ_c^2) which we have found to be .000090 ($= .0095^2$). The colorimetric error is thus only about three percent of the total error in this case.

In comparisons of sample one with standard, experimental error is involved only once. Thus the colorimetric error should constitute nearly twice as great a proportion of the total as above, viz., $\sigma_c^2/\sigma_e^2 + \sigma_c^2$, or 5.9 percent, provided the colorimetric error is the same (on the appropriate scale) as in the comparison with the other sample.

A second test can be obtained from the correlation of the mean direct readings of one sample against the other with the corresponding ratio of the mean readings of each against the standard. If there were no colorimetric error, the correlation (r) would necessarily be perfect. The actual value of r in the yellow series was $.89 \pm .02$. The portion of the total error due to colorimetry can be found as follows. A direct reading of one sample against the other, deviates in a particular case because of the errors in the preparation of both solutions and also because of *one* error of colorimetry. The ratio of the mean readings against standard deviates from one because of the same errors in preparation plus *two* errors of colorimetry. Let $\sigma_{c_1}^2$ equal the squared standard error of colorimetry in this case. It may differ from σ_c^2 since the samples may vary considerably from standard in concentration. The degree of determination by errors of preparation is

$$\frac{2\sigma_e^2}{2\sigma_e^2 + \sigma_c^2} = .97 \text{ in the former case and } \frac{2\sigma_e^2}{2\sigma_e^2 + 2\sigma_{c_1}^2} \text{ in the latter. The cor-}$$

relation coefficient may be equated to the product of the square roots of

these expressions which are the path coefficients measuring the contributions of the common factors. This yields $2\sigma_e^2/2\sigma_e^2 + 2\sigma_{c_1}^2 = .82$. The portion of the variance due to experimental error in a comparison of one sample with standard should agree ($\sigma_e^2/\sigma_e^2 + \sigma_{c_1}^2$) giving 18 percent as the portion of the variance in this case due to colorimetry. This is about three times as large as the estimate derived from the readings of the samples against each other and seems to indicate that errors of colorimetry are much greater in comparisons with standard. Nevertheless the colorimetric error is still a minor portion of the total error. The experimental error in preparing one sample may be estimated as .00144 ($-\frac{1}{2} .00296 - .00009$). Since $\sigma_e^2/\sigma_e^2 + \sigma_{c_1}^2 = .82$, the total error in grading one sample with the standard

($\sigma_e^2 + \sigma_{c_1}^2$) may be estimated as $.00176 \left(= \frac{.00144}{.82} \right)$. The standard deviation of total errors for one sample is thus about 4.2 percent ($= \sqrt{.00176}$).

The standard deviation for the average of two samples is thus about 3.0 percent $\left(= \frac{4.2}{\sqrt{2}} \right)$.

A comparable statistical analysis for the sepia genotypes (90 experiments run in duplicate and including grades 10-21) yielded a total variance of .00129 for the means of sets of direct readings of one sample against the other. The variance within the sets of ten readings was .00065 and thus the variance of means, due to colorimetry, was .000065 or about 5.0 percent of the total. This would imply about 9.5 percent determination of the total squared error by colorimetric errors in the case of the reading of one sample against standard, if the colorimetric errors remain the same. As before, however, analysis of the correlation between the direct readings, sample two against sample one, and the ratio of the readings against standard, indicates a larger average colorimetric error in the readings against standard. The correlation coefficient was $.86 \pm .03$ in this case. This

gives 78 percent $\left(= \frac{.86^2}{.95} \right)$ as the estimated portion of the variance due to

experimental errors, in comparisons with standard, and 22 percent (instead of 9.5 percent) as the portion due to colorimetric errors. The total error in a comparison of one sample with standard may be estimated as

$.00079 \left(= \frac{.000618}{.78} \right)$. The standard deviation of total errors for one sample

is thus about 2.8 percent ($= \sqrt{.00079}$) and the standard deviation for the average of two samples is about 2.0 percent. It should be pointed out that

standard deviations of such a size are a little low for the method as a whole. This is accounted for by the following considerations. All experiments in which the two samples from the same animal were grossly different were repeated. In eight of the nine cases in which this procedure was followed the solutions of the second gave readings within a ten percent range of each other and were, therefore, regarded as reflecting a more accurate value for the animal under experimentation. The most probable source of gross error in such cases was loss of pigment during decantation of the hydrolysate or wash water.

The yellow series

The colorimetric values for the yellow series are given by grade in table 1 and by genotype in table 2 and figure 1. The standard errors in tables 1 and

TABLE 1

A comparison of the colorimetric values for grades of yellow with those estimated by Russell.

GRADE	PRESENT DATA					RUSSELL'S DATA		
	NO. OF ANIMALS	MEAN CV	σ	GENERALIZED σ	S.E.	NO. OF ANIMALS	MEAN CV	GENERALIZED S.E.
12	3	1.849	.156	.348	$\pm .201$	1	2.654	$\pm .667$
11	6	1.516	.258	.285	.117	9	3.020	.253
10	13	1.460	.276	.275	.076	15	2.872	.186
9	6	1.164	.167	.219	.090	2	2.237	.397
8	5	0.848	.104	.160	.071	6	1.403	.144
7	27	0.618	.101	.116	.022	34	1.039	.045
6	10	0.544	.112	.102	.032	12	0.778	.050
5	7	0.413	.104	.078	.029	17	0.627	.038
4	13	0.235	.054	.044	.012	22	0.552	.030
3	7	0.173	.054	.035	.013	12	0.357	.026
2	2	0.133	.019	.025	.018	4	0.239	.030
1	3	0.064	.015	.020	.012	1	0.122	.031
0	4	0.057	.005	.017	$\pm .008$	3	0.120	$\pm .017$

2 were calculated in the usual manner but from a generalized standard deviation secured by multiplying the mean for each grade or genotype, as the case might be, by the weighted mean coefficient of variability for all grades or genotypes (weight $\times n - 1$). This procedure was followed in order to find a reasonably reliable measure of variability for groups in which the numbers were so small that standard errors calculated from the actual standard deviations would have little meaning. In grades 10, 7 and 4, classes which occur most frequently, and which, therefore, may be assumed to show the most reliable means, the generalized standard deviation varies only slightly from that calculated directly. The values are reduced in

grades 3-6 but in all other instances they are about the same or larger than the conventional standard deviations.

The uncertainty of the means for very high grades (12, 11, 10) is probably partly due to the fact that animals of high intensity do not hold their original color as do those of lower grades (exclusive of *ff* genotypes). After the data on grades 12 and 11 had been secured, the small samples of hair

TABLE 2

A comparison of the colorimetric values of yellow genotypes with those estimated by Russell.

PRESENT DATA							RUSSELL'S DATA			
<i>eeF-</i>							<i>eeF-</i>			
C SE- RIES	NO. OF ANI- MALS	MEAN GRADE	MEAN CV	σ	GENER- ALIZED σ	S.E.	NO. OF ANIMALS	MEAN CV	S.E.	RUSSELL'S TRANS- FORMED CV
<i>C^k</i>	2	11.5	1.643	.545	.360	±.255	..		±.....	
<i>C^d</i>	9	10.0	1.422	.273	.312	.104	..			
<i>C^r</i>	3	9.3	1.225	.208	.269	.155	..			
<i>C^a</i>	6	10.3	1.530	.301	.335	.137	..			
<i>C^{-*}</i>	28	10.2	1.450	.300	.318	.060	27	2.866	.114	1.571
<i>c^kc^k</i>	8	6.8	.502	.069	.110	.039	10	.949	.055	.474
<i>c^kc^d</i>	1	7.0	.611		.134	.134	6	1.031	.150	.521
<i>c^kc^r</i>	1	4.0	.183		.040	.040	13	.616	.048	.284
<i>c^kc^a</i>	4	4.0	.513	.039	.047	.023	4	.489	.025	.211
<i>c^dc^d</i>	21	6.8	.676	.129	.148	.032	23	1.150	.082	.589
<i>c^dc^r</i>	10	4.6	.365	.118	.080	.025	19	.593	.046	.271
<i>c^dc^a</i>	1	4.0	.279		.061	.061	18	.484	.036	.208
<i>c^rc^r</i>	1	0.0	.054		.012	.012	2	.133	.017	.007
<i>c^rc^a</i>	2	0.0	.058		.009	.009	..			
<i>c^ac^a</i>	1	0.0	.058		.013	.013	..			
<i>eeff</i>							<i>eeff</i>			
<i>C⁻</i>	11	6.8	.636	.138	.139	.042	7	.888	.053	.439
<i>c^kc^k</i>	2	1.0	.057	.011	.012	.009	..			
<i>c^dc^d</i>	10	2.7	.150	.051	.033	±.010	10	.280	±.031	.092

* Total includes genotypes in which the second allele was unknown.

left from the experiments were examined and compared with the standard pelts. Although it is difficult to evaluate small wisps of hair, there was no doubt whatever that all of the samples of grade 12 and three of grade 11 had faded noticeably. These observations, therefore, indicate that the lighter intensities of adult animals are foreshadowed in some cases, at least, by a fading of the color as early as three to four weeks of age.

It will be noted that in harmony with the previous observations of

$$\log (CV-.057)=\bar{8}.735+.138G \text{ (not weighted)}$$

$$\log (CV-.057)=\bar{8}.812+.132G \pm .0039 \text{ (weighted)}$$

and those quoted by RUSSELL

$$\log (CV-.12)=\bar{8}.987+.136G \text{ (not weighted)}$$

$$\log (CV-.12)=\bar{9}.032+.135G \pm .0046 \text{ (weighted)}.$$

If RUSSELL's line be made to intersect the author's at grade 7, her equation becomes

$$\log (CV-.12)=\bar{8}.779+.135G$$

It is obvious that neither weighting nor the use of a series of standards in place of the single one of grade 7 used by Russell has resulted in any substantial differences. Slightly significant departures from linearity (weighted line) are indicated by *t* values of -2.9 , -3.1 , $+3.0$, -2.7 , -2.8 for grades 3, 4, 6, 11, 12 respectively, and also by grouping of the signs for *t* in grades 1-4, 5-10, 11-12.

The tabulations according to genotype (table 2 and figure 1) are of interest in evaluating the dominance relations of the *C* series. In order to make the scales of RUSSELL and the author comparable, the corrected values as given by her must be multiplied by .572, the factor which was found to make her value for grade 7 as given by the line coincide with that of the author. Table 3 gives the percentage of the intensity of type

TABLE 3

Percentage of the intensity of C-F- in other gene combinations.

GENOTYPE	PRESENT DATA		RUSSELL'S DATA	
	NO. OF ANIMALS	PERCENT	NO. OF ANIMALS	PERCENT
<i>C-F-</i>	28	100.0	27	100.0
<i>c^kc^kF-</i>	8	32.0	11	30.2
<i>c^kc^dF-</i>	1	39.8	6	33.2
<i>c^kc^rF-</i>	1	9.0	13	18.1
<i>c^kc^aF-</i>	4	11.2	4	13.6
<i>c^dc^dF-</i>	21	44.4	23	37.5
<i>c^dc^rF-</i>	10	22.2	19	17.3
<i>c^dc^aF-</i>	1	15.9	8	13.2
<i>c^ac^ac^aF-*</i>	4	0.0	2	0.0
<i>C-ff</i>	11	41.6	7	27.9
<i>c^kc^kff</i>	2	0.0	0	
<i>c^dc^dff</i>	10	6.7	10	5.9

* The double superscripts used throughout this paper signify all of the possible gene combinations indicated by the letters. For example, *ee c^ac^aF-* = *ee c^rc^rF-*, *ee c^rc^aF-*, *ee c^ac^aF-*, all phenotypically white; *ee c^dc^aff* = *ee c^dc^rff*, *ee c^dc^aff*, phenotypically white; *ee c^dc^aF-* = *ee c^dc^rF-*, *ee c^dc^aF-*, phenotypically cream.

($C-F-$) found in other combinations (after subtraction of the correction factor .057).

The author's determinations are in essential agreement with the previous observations of RUSSELL and also with expectations based on WRIGHT's grades. The evidence indicates that C is dominant over its lower alleles, that $c^k c^k$ and $c^d c^d$ reduce the intensity markedly (to 32 and 44 percent of $C-F-$, respectively, after subtraction of the correction factor .057), that with $c^r c^r$ and $c^a c^a$ the threshold for the production of yellow has not been attained. The readings for $c^k c^k$, $c^k c^d$, $c^d c^d$ agree with all previous

TABLE 4

A comparison of the colorimetric and permanganate numbers (PN) for sepias tabulated by grade.

GRADE	NO. OF ANIMALS	PRESENT DATA				RUSSELL'S DATA		
		MEAN CV	σ	GENERAL-IZED σ	S.E.	NO. OF ANIMALS	MEAN* PN	S.E.
21	18	1.440	.212	.214	$\pm .050$	9	144	± 7
20	13	1.297	.176	.192	.053	5	115	7
19	17	1.011	.159	.150	.036	6	120	7
18	10	.894	.134	.133	.042	1	103	15
17	9	.755	.163	.112	.037	2	96	10
16	4	.782	.066	.116	.058	1	99	15
15	7	.609	.059	.090	.034	6	75	5
14	6	.526	.101	.078	.032	2	64	6
13	2	.478	.021	.071	.051	3	56	6
12	1	.347		.052	.052	2	62	7
11	2	.330	.039	.049	.035			
10	1	.390		.058	$\pm .058$	..	...	$\pm$

* PN is the abbreviation for "permanganate number" as defined by RUSSELL

(PN) = cc $\text{KMnO}_4 \times \text{NKMnO}_4 \times 100/\text{grams hair}$.

The values given in the table are uncorrected. To secure the corrected mean in each case subtract 12, the estimated value for adsorbed impurities.

results in falling within a relatively narrow range (weighted averages: present data: 40.9 percent of $C-F-$, RUSSELL's data 34.8 percent). There are, however, differences: $c^d c^d$ is significantly more intense than $c^k c^k$ ($t = 4.7$). Similarly $c^k c^r$, $c^k c^a$, $c^d c^r$, $c^d c^a$ fall within narrow limits, but at a level somewhat less than half that of the preceding genotypes (weighted average: present data 45.3 percent of $c^{kd} c^{kd}$, RUSSELL's data 47.1 percent). Again the combinations involving c^d are significantly more intense than those involving c^k ($t = 4.0$). The apparently greater intensity of c^k combinations is contrary to the slightly lower intensities observed by WRIGHT in the stocks on which he published in 1927. Further study will be necessary to determine how far these differences are due to real variations in

the effects of the genes and how far to differences in associated minor factors.

The drastic effect of ff is revealed by readings from $eeC-F-$, $eeC-ff$; $ee c^d c^d F-$, $ee c^d c^d ff$. In the presence of C , ff has about 42 percent (present data) or 28 percent (RUSSELL's data) as much pigment as F . In the presence of $c^d c^d$, ff has about 15 percent (present data) or 16 percent

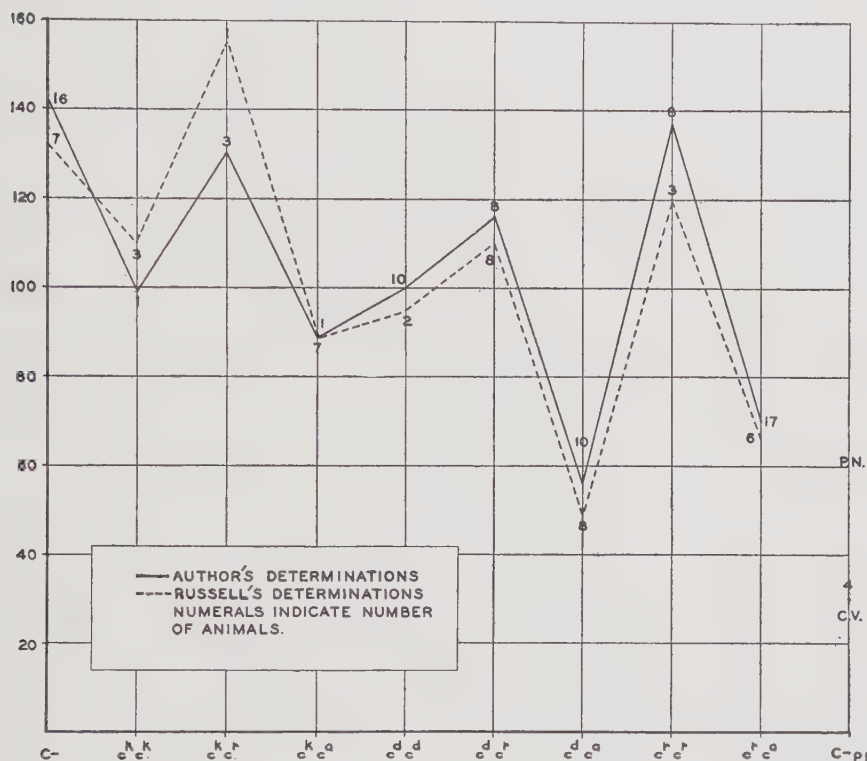


FIGURE 2.—A comparison of the colorimetric values and permanganate numbers for sepia genotypes. Scales are made comparable by multiplying the corrected permanganate numbers by .0101.

(RUSSELL's data) as much pigment as F . The disproportionately larger effect in $c^d c^d$ foreshadows the complete elimination of yellow in $c^d c^a ff$ in contrast with the considerable quantity present in $c^d c^a F-$.

The sepia series

The data for the sepia series are given by grade in table 4 and for genotype in table 5 and figure 2. The same procedure was followed in calculating the standard errors as that previously described for the yellow series. Since there are probably real differences in the coefficients of variability of genotypes (for example, $c^r c^a$ extending over 7-10 grades in comparison

to $c^k c^{ka}$ which cover only four grades, WRIGHT 1925) it is probably not as legitimate to generalize the standard deviations of genotypes as grades. In all cases standard deviations calculated in the usual manner are included together with the generalized standard deviations.

The determinations by grade show a consistent drop from grade to grade except for 16 and 10 in which the numbers were small. As with the grades

TABLE 5

A comparison of the colorimetric and permanganate number (PN) for sepia tabulated by genotype.

PRESENT DATA							RUSSELL'S DATA			
<i>E-P- DARK-EYED SEPIAS</i>							<i>E-P- DARK-EYED SEPIAS</i>			
C SERIES	NO. OF ANI-MALS	MEAN GRADE	MEAN CV	σ	GENERAL-IZED	S.E.	NO. OF ANI-MALS	MEAN* PN	S.E.	TRANS-FORMED PN
<i>CC</i>	1	19.00	1.329		.233	$\pm .233$	..	...	$\pm ..$	
<i>Cc^d</i>	5	20.80	1.358	.221	.238	.076	..	...	..	
<i>Cc^a</i>	10	21.00	1.561	.171	.274	.123	..	...	..	
<i>C-</i>	16	20.75	1.420	.216	.249	.062	7	143	9	1.323
<i>c^k c^k</i>	1	21.00	.994		.174	.174	3	120	12	1.091
<i>c^k c^r</i>	3	20.00	1.297	.250	.228	.131	1	166	26	1.555
<i>c^k c^a</i>	7	18.86	.885	.098	.155	.059	1	100	17	.889
<i>c^d c^d</i>	10	18.40	.996	.160	.175	.055	2	106	13	.949
<i>c^d c^r</i>	8	19.25	1.164	.284	.204	.072	8	121	7	1.101
<i>c^d c^a</i>	10	14.20	.562	.106	.099	.060	8	60	4	.485
<i>c^r c^r</i>	8	20.00	1.368	.194	.240	.084	3	131	12	1.202
<i>c^r c^a</i>	17	16.18	.699	.156	.123	.030	6	77	6	.657
<i>E-pp PINK-EYED SEPIAS</i>							<i>E-pp PINK-EYED SEPIAS</i>			
<i>C-</i>	4	11.00	.349	.036	.060	$\pm .030$	1	76	± 14	.646

* The values given are uncorrected. To secure the corrected mean in each case subtract 12, the estimated value for adsorbed impurities.

of yellow, it is also obvious here that the absolute decrements are large at the upper end of the scale and comparatively small at the lower end. The same methods were therefore applied in calculating the line which best fits the points determined by the logarithms of the colorimetric values plotted against grade (G). The slopes found for the colorimetric values and RUSSELL's titrations are shown in the following equations:

$$\log CV = 8.893 + .060G \text{ (unweighted, grades 21-13)}$$

$$\log CV = 8.849 + .062G \pm .0030 \text{ (weighted, grades 21-13)}$$

$$\log CV = 8.865 + .061G \pm .0025 \text{ (weighted, grades 21-10)}$$

$$\log (PN - 12) = .997 + .053G \pm .0048 \text{ (weighted, grades 21-13)}$$

$$\log (PN - 12) = .953 + .056G \pm .0025 \text{ (weighted, grades 21-2).}$$

The line given by RUSSELL's weighted equation for grades 21-13 was transformed so as to intersect the author's line at grade 17. RUSSELL's line then became:

$$\log (PN - .12) = 9.002 + .053G.$$

Evidently the use of weights makes substantially no difference to the slopes in the first two equations. RUSSELL's data contain no determinations for grades 10 and 11 and only two for grade 12. Thus the most suitable equations for comparison are those for grades 21-13, although the

TABLE 6

Percentage of the intensity of C-P- in other gene combinations.

GENOTYPE	PRESENT DATA		RUSSELL'S DATA	
	NO. OF ANIMALS	PERCENT	NO. OF ANIMALS	PERCENT
<i>C-P-</i>	16	100.0	7	100.0
<i>c^kc^kP-</i>	1	70.0	3	82.5
<i>c^kc^rP-</i>	3	91.3	1	117.5
<i>c^kc^aP-</i>	7	62.3	1	67.2
<i>c^dc^dP-</i>	10	70.1	2	71.7
<i>c^dc^rP-</i>	8	82.0	8	83.2
<i>c^dc^aP-</i>	10	39.6	8	36.7
<i>c^rc^rP-</i>	8	96.3	3	90.9
<i>c^rc^aP-</i>	17	49.2	6	49.6
<i>C-pp</i>	4	24.6	1	49.7

line for the colorimetric values is sufficiently stable so that inclusion of grades 10, 11, 12 does not alter the slope appreciably. The difference in slope constants .062 and .053 for grades 21-13 as given by the colorimetric and permanganate methods cannot be regarded as significant ($t = 1.6$). The colorimetric equation shows no significant departure from linearity since a comparison of calculated and observed values gives estimates of 2.4 or less for t and the signs are not clustered. There is, of course, no equation which includes colorimetric readings for grades 2-12 to compare with RUSSELL's in as much as the colorimetric method was not applicable to grades 2-9.

The close agreement between the permanganate and colorimetric values, especially in genotypes in which the numbers were fairly large, is shown in tables 5 and 6 and figure 2. The last column in table 5 gives the values of RUSSELL, transformed by multiplying each of her entries (corrected) by .0101, the factor found to make the lines of RUSSELL and the author coincide at grade 17. From tables 4, 5 and 6 and figure 2 we see evidence for the complete dominance of *C* over its lower alleles, a rather high degree of dominance of *c^k* over *c^a* ($c^k c^a = .89c^k c^k$) and approximate intermediacy of *c^d c^a* and *c^r c^a* compared to *c^d c^d* and *c^r c^r* ($c^d c^a = .56 c^d c^d$, $c^r c^a = .51 c^r c^r$).

Determinations for $c^d c^r$ are elevated when compared to $c^d c^d$ as are also $c^k c^r$ when compared to $c^k c^k$, $c^r c^r$ compared to $c^d c^d$, and $c^r c^a$ compared to $c^d c^a$ (within the limits indicated by the standard errors). These data are confirmatory of previous observations made by WRIGHT on the basis of grade alone and also of RUSSELL's results. The most serious discrepancy between the permanganate and colorimetric values is in the pink-eyed sepias of constitution $C-$ in which the ratios of $C-P-$ to $C-pp$ are approximately 2 and 4 respectively. The numbers are small, one and four, but the colorimetric value would appear to give the more reliable estimate as judged by the relatively small standard deviation and the appearance of the hair as determined by the grades assigned.

SOURCES OF VARIABILITY

The data indicate a rather wide variability within both grade and genotype. At first sight one might expect such fluctuations within genotype on the basis of a consideration of the range of grade within genotypes (WRIGHT 1927), but comparatively small differences within grades since all animals of a given grade look alike. A careful examination of the hair samples, however, indicates differences in the distribution of pigment from tip to base within grade. These differences are less developed in the short hair at birth (when the grades were assigned) than at three to four weeks. No attempt has been made to take the basal color into account in grading. Causes for this variation in gradient are as yet unanalyzed although there is evidence that modifying genetic factors play a role. In addition to this source of variability, subjective errors of grading are occasionally responsible for differences of a grade.

The reasons for the instability of readings within genotype are not wholly known, but some suggestions as to the sources of variation can be given. The non-isogeneity of the hereditary background certainly suggests one possible factor; the role of environmental agents is also of probable importance. There is no doubt about the presence of at least one modifying genetic factor which reduces the intensity of both yellow and sepia (unpublished data from a stock not included in the present investigation but derived from the lighter sepias and yellows of the stock used). The large amount of literature on temperature and plucking with its consequent cooling effect shows that the deposit of pigment may be modified by these factors. The fact that the standard pelts undergo some fading of color with time and exposure to light while being used points to light as another controlling source of variability. Competition for food within the cages may also help to account for differences within litter-mates of the same genetic constitution. Here it is appropriate to note the works of HARTWELL (1923) and HAYDAK (1935) which have shown that the intensity of the color in

black rats could be reduced by restrictions of diet. The intensity could be restored in part at least by resumption of the stock diets or others containing adequate sources of proteins such as tyrosine and tryptophane, and other food constituents. A consideration of all of the above factors leaves little wonder that the colorimetric and permanganate readings show comparatively large fluctuations.

SUMMARY

1. Colorimetric determinations of the amount of yellow pigment in the hair of known genotypes of guinea-pig have confirmed and extended the previous evaluations by RUSSELL.

2. A colorimetric method was developed by which it has been possible to secure measurements of sepia solutions for grades 10-21. These include black-eyed sepias and pink-eyed sepias of constitution $C-$. Determinations by this method closely parallel the findings of RUSSELL who used a very different method. (Titration of pigment with potassium permanganate.)

3. An analysis has shown that the total experimental error for the yellow series (one set of ten readings) was 4.2 percent and for the sepia series 2.8 percent. About 18 percent of the total error was due to colorimetry in the case of the yellow series, and 22 percent in the sepia series.

4. The colorimetric determinations indicate that C is completely dominant over its lower alleles in both the yellow and sepia series, and that $c^k c^a$, $c^k c^r$; $c^d c^a$, $c^d c^r$ compounds in the yellow series show somewhat less than half as much pigment as $c^k c^k$ and $c^d c^d$ respectively. Genotypes $c^r c^r$, $c^r c^a$, $c^a c^a$ are pure white in place of yellow. The drastic reduction of yellow by ff is shown to be disproportionately larger in $c^d c^d$ than in $C-$. In the sepia series, c^k shows a fairly high degree of dominance over c^a , but little or no dominance is exhibited by c^d and c^r over c^a ($c^a c^a$, white). All comparisons show that c^r produces more sepia than c^d . Genotypes of $C-P-$ constitution gave readings which were roughly four times those of $C-p p$.

5. The determinations for grade and genotype in both series showed considerable variability. Suggestions as to factors which may control these fluctuations are given.

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